

Kepone: Toxicity and Bioaccumulation in
Blue Crabs

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Kepone®: Toxicity and Bioaccumulation in Blue Crabs¹

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ABSTRACT: Two long-term studies were conducted to determine toxicity, uptake and depuration of Kepone in blue crabs (*Callinectes sapidus*). In the first, Kepone was administered to crabs in seawater (0.03 or 0.3 µg Kepone/l) or food (eastern oyster, *Crassostrea virginica*, containing 0.25 µg/g Kepone). Uptake of Kepone in 28 days was primarily through the contaminated oysters. When these crabs were held in Kepone-free seawater and fed Kepone-free oysters for 28 days, no loss of the insecticide was evident. There were adverse effects on molting and survival in crabs fed oysters that contained 0.25 µg/g Kepone.

A second study was conducted to determine: (1) the depuration of Kepone over a 90-day period in blue crabs fed oysters from the James River, Virginia (containing 0.15 µg/g Kepone); and (2) the effects of Kepone on molting and survival of blue crabs fed James River oysters or laboratory-contaminated oysters that contained 0.15 or 1.9 µg/g Kepone. Crabs fed Kepone-contaminated oysters followed by a diet of Kepone-free oysters for 90 days had detectable concentrations of the insecticide in tissues. Also, blue crabs that ate oysters containing Kepone in concentrations similar to those found in oysters from the James River, died or molted less frequently than crabs fed Kepone-free oyster meats.

Introduction

Contamination of Virginia's James River estuary by Kepone in the late 1960's and early 1970's, and its transport into Chesapeake Bay, raised questions about the chemical's persistence in and effects on the area's estuarine biota. Kepone residues in James River biota, in particular blue crabs (*Callinectes sapidus* Rathbun), bluefish (*Pomatomus saltatrix* Linnaeus), and eastern oysters (*Crassostrea virginica* Gmelin), were found by the U.S. Food and Drug Administration (FDA) to be sufficiently high to limit the commercial harvesting of these and other species captured in the estuary.

Kepone residues in James River blue crabs coincided with a decrease in commercial crab landings. Residues in males was 0.81 µg/g; in

females, 0.19 µg/g (Bender et al. 1977). Total blue crab catch from the James River indicated a 90% decline from 1972 through 1975 (U.S. Dept. Commerce 1968-1975).

Laboratory exposures of blue crabs in seawater to Kepone indicated relatively low acute toxicity and bioconcentration (Schimmel and Wilson 1977). This, in contrast to James River residue data, suggested that the major route of Kepone entry in crabs may be through contaminated food.

In this paper, we report the results of two long-term studies to determine: (1) uptake and depuration rates of Kepone in blue crabs exposed to the insecticide in seawater and food (eastern oysters); and (2) effects of Kepone in food on molting and survival of blue crabs.

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¹ Contribution No. 349, Environmental Research Laboratory, Gulf Breeze.

Protection Agency's (EPA) Environmental Research Laboratory, Athens, Georgia, for analyses of heavy metals in oyster tissues.

Materials and Methods

TEST ANIMALS

Blue crabs for both studies were collected from Santa Rosa Sound, Florida; oysters, used as food for blue crabs, were collected either from Santa Rosa Sound (local oysters) or from Wreck Shoals in the James River estuary, Virginia. Eastern oysters were appropriate food in these bioaccumulation studies because: (1) oysters bioconcentrate Kepone under laboratory conditions (Bahner et al. 1977); (2) oysters contaminated with Kepone have been reported from the James River (Bender et al. 1977); and (3) oysters are a natural food of blue crabs (Anonymous 1941; Loosanoff 1948; Lunz 1947; Menzel and Hopkins 1956; and Menzel and Nichy 1958). Approximately 95% of the crabs collected were *Callinectes sapidus*; the remaining, *C. similis* (Williams). No effort was made to separate the latter species because of the limited numbers of animals available and the need to avoid excessive handling. Locally collected oysters (Santa Rosa Sound) and blue crabs contained no Kepone detectable by gas chromatographic analysis. Blue crabs were acclimated to laboratory conditions for at least 14 days prior to testing.

STUDY NO. 1

Approximately 450 juvenile blue crabs (19–49 mm carapace width, $\bar{x}$ = 36.7 mm) were collected from Santa Rosa Sound, in July 1976. During acclimation, crabs were fed meats of eastern oysters from Santa Rosa Sound *ad libitum*.

A 56-day bioconcentration (uptake directly from the exposure water) and bioaccumulation (uptake primarily from ingested food) study was conducted, using the diluter described by Schimmel et al. (1977). Seawater was pumped from Santa Rosa Sound through a sand filter and into a reservoir in the laboratory. It was then heated to a mean temperature of 22.1°C (range, 20.0 to 23.5°C); salinity was allowed to vary with sound conditions (range 20.0 to 31.0 ‰; $\bar{x}$ = 25.5 ‰). From the

reservoir, seawater was pumped to the diluter mixing box by Lambda® pumps. The diluter provided two Kepone concentrations in seawater (0.03 µg/l and 0.3 µg/l, nominal concentrations), each concentration duplicated, as well as seawater free of Kepone, also duplicated. Six exposure aquaria measured 46 cm × 71 cm × 26 cm (volume = 86 l), and the diluter delivered one liter to each aquarium each cycle. There was an average of 300 cycles each day (10 turnover volumes). Fifty-six blue crabs were placed simply in individual compartments (6.5 cm × 4.7 cm × 10.0 cm) in each aquarium. At least 12 holes (6 mm diameter) were drilled in each compartment to provide adequate circulation.

The 56-day study was divided into a 28-day uptake period and a 28-day depuration period. In the uptake period, Kepone was administered to the crabs (1) in the water delivered to the experimental aquaria, and (2) in contaminated eastern oysters as a food source. Adult oysters were contaminated by exposing them to 0.1 µg/l Kepone for 42 hours; afterwards oyster meats were removed from their shells, pooled, and analyzed quantitatively for Kepone content. Approximately 0.5 g of the oysters, contaminated with 0.25 µg/g Kepone, were fed to the crabs twice each week in a control aquarium, and to crabs exposed either to the 0.03 µg/l or 0.3 µg/l. Approximately 0.5 g of uncontaminated, Santa Rosa Sound oysters (< 0.02 µg/g Kepone) were fed to crabs in the remaining control and experimental aquaria (Table 1). Two crabs were sampled from each aquarium during the uptake portion of the test at 1 day, 2 days, and, after the second day, twice each week to the end of the 28-day exposure period. Identical sampling intervals were used for the 28-day depuration portion of the study. When sampled, each crab was dissected into muscle and remaining tissues including exoskeleton. Muscle tissue was obtained from the thoracic region and contained small amounts of chitin. Remaining tissues consisted of whole crab not included in the muscle tissue sample. Molting and mortality of crabs were monitored daily throughout the study.

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STUDY NO. 2

Approximately 580 juvenile blue crabs (9 to 42 mm carapace width, $\bar{x}$ = 28 mm) and approximately 1000 adult eastern oysters were collected from Santa Rosa Sound in February and March, 1977.

Uncontaminated local oysters, the control food, were removed from their shells, cut into 0.5 g portions, and frozen.

Two hundred local, uncontaminated oysters were placed in an aquarium and exposed to 1.0 $\mu\text{g/l}$ Kepone for 20 hours to obtain an average of 1.9 $\mu\text{g/g}$ Kepone in their tissues. Another 200 oysters were exposed to 0.1 $\mu\text{g/l}$ to obtain an average of 0.15 $\mu\text{g/g}$ Kepone. Oysters from each aquarium were then cut into 0.5 g pieces and frozen.

Additional eastern oysters were collected in the James River (near Wreck Shoals) in March 1977 by the staff of the Virginia Institute of Marine Science and shipped by air freight to the Gulf Breeze Environmental

Research Laboratory. Gas chromatographic analysis of pooled oysters showed a Kepone concentration of 0.15 $\mu\text{g/g}$ wet weight. These oysters were also cut into 0.5 g pieces and frozen.

Study No. 2 with blue crabs was conducted to determine: (1) the effects of Kepone on molting and survival; and (2) the rate of depuration of Kepone beyond the 28-day period tested in Study No. 1. The same exposure apparatus used in Study No. 1 was used except that no Kepone was administered in seawater. Six aquaria were used, each containing 68 blue crabs (held in individual compartments as in the previous study). Crabs in aquaria Nos. 1 and 4 were used to determine uptake and depuration of Kepone administered through their food; those in aquaria Nos. 2, 3, 5, and 6 were observed for effects on molting or survival (Table 2). Crabs in aquarium No. 1 were fed 0.5 g of Kepone-free oysters twice each week. Crabs were sampled twice each week for 28 days, three per sample, for Kepone residue analyses; thereafter, crabs were sampled less frequently for an additional 90 days. Crabs were dissected into muscle and remaining tissues, as in the first study.

CHEMICAL METHODS

Methods of chemical analyses of Kepone were those of Schimmel and Wilson (1977). The average recovery rate of Kepone from fortified tissue was 87%; from water, 85%. Residue concentrations were calculated on a wet-weight basis without correction for percentage recovery. All samples were fortified with an internal standard (dichlorobenzophenone) prior to analysis to evaluate the integrity of the results.

TABLE 1. Concentrations of Kepone used in Study No. 1 for 28-day exposure of blue crabs (*Callinectes* spp.) to the insecticide in seawater or in food (eastern oyster, *Crassostrea virginica*).

Aquarium	Kepone ¹	
	Water ($\mu\text{g/l}$)	Food ($\mu\text{g/l}$)
1	ND ²	0.25
2	0.03	ND
3	0.03	0.25
4	0.3	0.25
5	0.3	ND
6	ND	ND

¹ Uptake period was followed by a 28-day depuration period in which no Kepone was administered in food or in seawater.

² ND = non-detectable: <0.02 $\mu\text{g/l}$ in water, <0.02 $\mu\text{g/g}$ in oyster tissue.

TABLE 2. Experimental design for a 56-day exposure of blue crabs (*Callinectes* spp.) to the insecticide, Kepone, in eastern oysters (*Crassostrea virginica*) (Study No. 2).

Aquarium	Source of Oysters	Concentration of Kepone in Oysters	Observation
		($\mu\text{g/g}$)	
1	Santa Rosa Sound, Florida	ND ¹	Molting and Survival
2	Santa Rosa Sound, Florida	ND	Uptake/Depuration
3	James River, Virginia	0.15	Molting and Survival
4	James River, Virginia	0.15	Uptake/Depuration
5	Santa Rosa Sound, Florida	0.15	Molting and Survival
6	Santa Rosa Sound, Florida	1.9	Molting and Survival

¹ ND = non-detectable: <0.02 $\mu\text{g/g}$ in tissues.

STATISTICAL METHODS

Blue crab molting data were analyzed by one-way analysis of variance followed by the Student-Newman-Keuls *post hoc* tests to determine significant differences ($\alpha = 0.05$); mortality data were analyzed by Student's *t* ($\alpha = 0.05$).

Results

STUDY NO. 1

Blue crabs fed oysters contaminated with 0.25 $\mu\text{g/g}$ Kepone accumulated the insecticide readily in their muscle and remaining tissues in 28 days. However, after 28 days in

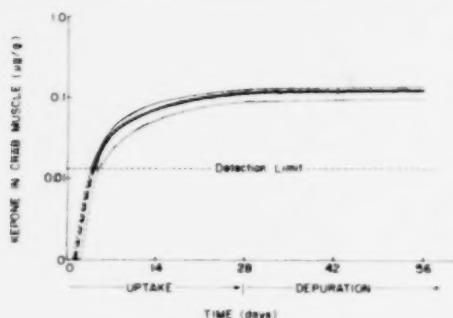


Fig. 1. Bioaccumulation of Kepone in muscle tissues of blue crabs (*Callinectes* spp.) fed oysters contaminated with 0.25 $\mu\text{g/g}$ of the insecticide for 28 days followed by a 28-day period for depuration. The uptake curve and the 95% confidence interval represented are a composite of three homogeneous curves representing uptake in crabs fed: (1) 0.25 $\mu\text{g/g}$ Kepone in oysters and control seawater, (2) 0.25 $\mu\text{g/g}$ Kepone in oysters and 0.03 $\mu\text{g/l}$ in seawater, and (3) 0.25 $\mu\text{g/g}$ in oysters and 0.3 $\mu\text{g/l}$ in seawater.

a Kepone-free environment, no depuration of the compound was evident (Fig. 1). When fed 0.25 $\mu\text{g/g}$ Kepone in oysters for four weeks, crabs accumulated the insecticide to an average of approximately 0.1 $\mu\text{g/g}$ in both their muscle and remaining tissues. Uptake of Kepone by crabs fed contaminated oysters only (aquarium No. 1) was nearly identical to that of crabs fed contaminated oysters and provided Kepone in seawater (aquaria Nos. 3 and 4), indicating that very little of the insecticide was taken in directly from water. No measurable concentrations of Kepone were detected in crabs from aquaria supplied with 0.3 $\mu\text{g/l}$ (0.08 $\mu\text{g/l}$ measured) or 0.03 $\mu\text{g/l}$ (0.014 $\mu\text{g/l}$ measured) Kepone (aquaria Nos. 5 and 2 respectively).

Blue crabs in all aquaria were monitored for molting and mortality throughout the 56-day test. Although no statistical tests could be applied to these data, we observed that crabs fed control oysters molted a total of 63 times, and 22 crabs died (Table 3). Crabs fed Kepone-contaminated oysters for 28 days, and control oysters for 28 additional days, had fewer molts ($n = 48$) and more deaths ($n = 49$) than crabs fed only control oysters. The data indicated that Kepone in crab food may reduce their ability to molt or survive. Therefore, we conducted Study No. 2 to investigate this possibility and to determine the depuration rate of Kepone in crabs over a longer period than in the previous study.

STUDY NO. 2

Blue crabs in aquarium No. 4 that were fed James River oysters (containing 0.15 $\mu\text{g/g}$

TABLE 3. Total molts and mortality of blue crabs (*Callinectes* spp.) in Kepone Study No. 1. Fifty-six crabs were placed in each aquarium at the beginning of the test.

Aquarium No.	Test Conditions	Molts	Mortality
1	Control water ¹ , 0.25 $\mu\text{g/g}$ Kepone in oyster	18	15
2	0.03 $\mu\text{g/l}$ Kepone in water; Control oysters ¹	20	6
3	0.03 $\mu\text{g/g}$ Kepone in water, 0.25 $\mu\text{g/g}$ Kepone in oysters	12	20
4	0.3 $\mu\text{g/l}$ Kepone in water, 0.25 $\mu\text{g/g}$ Kepone in oysters	18	14
5	0.3 $\mu\text{g/l}$ Kepone in water; Control oysters	22	9
6	Control water, Control oysters	21	7

¹ Control water, <0.02 $\mu\text{g/l}$ Kepone; Control oysters, <0.02 $\mu\text{g/g}$ Kepone

Kepone) accumulated the insecticide in muscle and whole-body tissues (muscle and remaining tissues) to a maximum of $0.069 \mu\text{g/g}$ in 28 days (Fig. 2). After a Kepone-free diet for 90 days, crabs showed some loss of the insecticide. However, $0.025 \mu\text{g/g}$ Kepone was detected in remaining tissues, and approximately $0.014 \mu\text{g/g}$ remained in muscle tissues. Some of this apparent loss of Kepone must be attributed to growth of the blue crabs. No length or weight measurements were made at the end of the 28-day exposure period, and consequently the exact growth could not be determined.

Molting by crabs fed James River oysters (aquarium No. 3, Kepone concentration = $0.15 \mu\text{g/g}$), oysters containing $0.15 \mu\text{g/g}$ Kepone (aquarium No. 5), or $1.9 \mu\text{g/g}$ Kepone (aquarium No. 6) was significantly reduced ($\alpha = 0.05$) compared with those fed control oysters (aquarium No. 1, Fig. 3). The average molts per crab in 56 days were: control, 1.4; $1.9 \mu\text{g/g}$ Kepone, 0.56; $0.15 \mu\text{g/g}$ Kepone, 0.97; and James River oysters, 0.48.

Percent mortality in crabs fed oysters containing $1.9 \mu\text{g/g}$ Kepone and those fed James River oysters was significantly greater ($\alpha = 0.05$) than those fed control oysters (Fig. 4). Blue crabs which ate oysters containing $1.9 \mu\text{g/g}$ Kepone exhibited extreme excitation, especially during feeding. In the advanced stages of Kepone poisoning, the crabs were generally lethargic for several days before death. No crabs in any other aquaria dis-

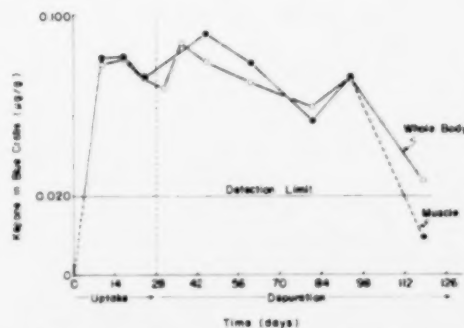


Fig. 2. The 28-day accumulation of Kepone by blue crabs (*Callinectes* spp.) fed James River oysters that contained $0.15 \mu\text{g/g}$ Kepone. In the depuration period, crabs were fed Kepone-free ($<0.02 \mu\text{g/g}$) oysters. Kepone residue for muscle on day 118 is estimated to be $0.014 \mu\text{g/g}$.

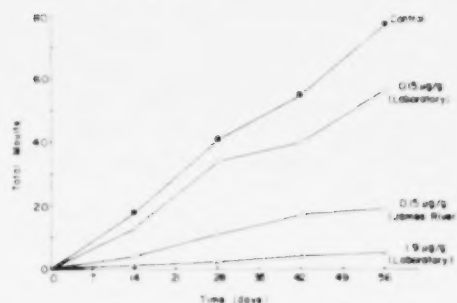


Fig. 3. Molting rate of blue crabs (*Callinectes* spp.) fed uncontaminated ($<0.02 \mu\text{g/g}$ Kepone) or Kepone contaminated eastern oysters (*Crassostrea virginica*) for 56 days on a flow-through bioassay. Molting rates are for crabs that survived the entire study in each test aquarium. The test conditions and numbers of surviving crabs are: control, 57 crabs; $0.15 \mu\text{g/g}$ Kepone, 58 crabs; James River, 40 crabs; and $1.9 \mu\text{g/g}$ Kepone, 9 crabs.

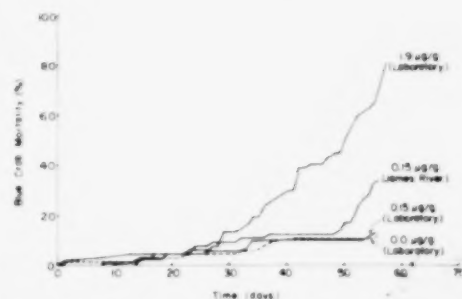


Fig. 4. Mortality of blue crabs (*Callinectes* spp.) fed uncontaminated ($<0.02 \mu\text{g/g}$ Kepone) or Kepone contaminated eastern oysters (*Crassostrea virginica*) for 56 days in a flow-through bioassay.

played these signs. Percent mortality of crabs fed oysters containing $0.15 \mu\text{g/g}$ Kepone was not significantly different from that of crabs fed control oysters.

Discussion

The uptake and depuration of Kepone by blue crabs in both studies help explain why relatively high Kepone residues are found in crabs from the James River, Virginia. No discernible depuration of Kepone in crab muscle or remaining tissues was evident in 28 days but some loss of Kepone was apparent beyond 28 days. However, detectable Kepone was present in tissues of crabs held 90 days in a Kepone-free environment. In the James

River, Bender et al. (1977) reported that average Kepone concentrations in estuarine vertebrates and invertebrates ranged from 0.09 to 2.0 $\mu\text{g/g}$. Many, if not all, of the species listed are included in the diet of the blue crab. Therefore, it is reasonable to conclude from our laboratory studies and James River field data that Kepone residues will persist in blue crab tissues as long as detectable concentrations remain in the food.

Blue crabs fed oysters containing 1.9 $\mu\text{g/g}$ Kepone exhibited signs of poisoning similar to crabs fed fish contaminated with 1.0 $\mu\text{g/g}$ of mirex (Lowe et al. 1971). Mirex is an organochlorine insecticide chemically similar to Kepone. Lowe et al. (1971) also reported that juvenile blue crabs exposed to 100 $\mu\text{g/l}$ mirex in flowing seawater for 96 hours showed no signs of poisoning. Schimmel and Wilson (1977) exposed blue crabs to Kepone for 96 hours at concentrations as high as 210 $\mu\text{g/l}$ and reported no significant mortality.

Blue crabs fed oysters from the James River (containing 0.15 $\mu\text{g/g}$ Kepone) died in significantly greater numbers and molted fewer times than those fed uncontaminated oysters; crabs fed oysters containing 0.15 $\mu\text{g/g}$ Kepone (by laboratory exposure) molted fewer times but did not die in significantly different numbers than those fed uncontaminated oysters. Reasons for the difference in mortality are unclear. However, it should be noted that a 0.15 $\mu\text{g/g}$ Kepone residue appears to be the threshold level for effect: blue crabs fed local oysters contaminated with 1.9 $\mu\text{g/g}$ Kepone suffered 80% mortality, and those fed local oysters contaminated with 0.25 $\mu\text{g/g}$ Kepone died in greater numbers than those fed control oysters. It is possible that the difference in mortality in the crabs fed James River oysters (0.15 $\mu\text{g/g}$) and those fed local, contaminated oysters (0.15 $\mu\text{g/g}$) may be due to a toxicant not detectable by our methods of chemical analysis or that our methods of exposure and duration were different than those of oysters from the James River. To investigate the possibility of some metal in the oysters, samples of local and James River oysters were sent to the EPA Environmental Research Laboratory, Athens, Georgia, for analysis. Results of these analyses (multielement analysis by plasma emission and spark source mass spectrometry)

indicated that oysters from the James River contained approximately 5 $\times$ more aluminum (70 $\mu\text{g/g}$), 10 $\times$ more copper (29 $\mu\text{g/g}$), and 9 $\times$ more zinc (415 $\mu\text{g/g}$) than local oysters. Concentrations of other metals in oysters from both areas were similar. We do not know the effects of these metals and Kepone on the survival of blue crabs.

Annual crab fishery statistics (U.S. Dept. Comm. 1968-1975) from the James River show that from 1968 to 1972 the commercial catch ranged from 691,000 kg to 1,211,000 kg ($\bar{x}$ = 899,000 kg), but from 1972 through 1975 the catch decreased more than 90% to 14,700 kg. (A moratorium on harvesting of blue crabs in the James River was in effect in 1976). The blue crab catch in the adjacent Rappahannock River estuary from 1968 through 1975 did not show a significant decline. From these catch data it appears that some perturbation is causing a particularly adverse effect on blue crab abundance in the James River estuary.

If the James River estuary had been affected by only one pollutant, it would have been comparatively easy to determine a cause and effect relationship with that pollutant and the decrease in the crab fishery. As in nearly all instances in the natural environment, this is not the case in the heavily industrialized James River area. However, based on our laboratory data, we believe that Kepone may be a factor in the decline of the James River crab fishery.

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Addendum to legend of Fig. 2: Seawater temperature averaged 25.5°C (range = 20 to 28°C); salinity averaged 23.3 ‰ (range 10 to 32 ‰).